Article

Not peer-reviewed version

First Evidence of Bovine Viral Diarrhea Virus Circulation in Libya

Hania Elkhoja , Imad Buishi , Emiliana Brocchi , Santina Grazioli , Abdusalam Mahmoud , Ibrahim Eldaghayes * , Abdunaser Dayhum *

Posted Date: 26 December 2023

doi: 10.20944/preprints202312.1947.v1

Keywords: BVDV; Risk factors; Sero-prevalence; Libya

Article

First Evidence of Bovine Viral Diarrhea Virus Circulation in Libya

Hania Elkhoja [1,2], Imad Buishi [2], Emiliana Brocchi [3], Santina Grazioli [3], Abdusalam Mahmoud [2], Ibrahim Eldaghayes [4,*] and Abdunaser Dayhum [2,*]

[1] National Center of Animal Health, Tripoli, Libya; hana131288@gmail.com
[2] Department of Preventive Medicine, Faculty of Veterinary Medicine, University of Tripoli, Tripoli, Libya; imadbuishi@hotmail.com (I.B.); abd.mahmoud@out.edu.ly (A.M.) ; adayhum@yahoo.com (A.D.)
[3] Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER), Brescia, Italy; emiliana.brocchi@gmail.com (E.B.) ; santina.grazioli@izsler.it (S.G.)
[4] Department of Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Tripoli, Tripoli, Libya; ibrahim.eldaghayes@vetmed.edu.ly (I.E.)
* Correspondence: ibrahim.eldaghayes@vetmed.edu.ly (I.E.); adayhum@yahoo.com (A.D.)

Simple Summary: Bovine Viral Diarrhea (BVD) is known to be endemic in Northen Africa. However, no data on the disease is available in Libya. One of the aims of this study was to provide data on BVD in Libya and to fill the gap in the region. Following collecting serum samples from cattle in Libya and testing these samples against BDV antibodies using ELISA test, the disease has been confirmed with higher percentage in older cattle. The results of this study give an idea that BVD virus is circulating in cattle in Libya.

Abstract: The epidemiological patterns of Bovine Viral Diarrhea (BVD) in North Africa and Mediterranean Basin (MB) are endemic with highly socio-economic impacts. The objective of the study was conducted to investigate the level of sero-prevalence of BVDV in Libya and the associated risk factor. A total of 1599 serum samples were collected from herds of cattle which belonging to seven Libyan regions. All sera were assayed by using screening ELISA assay for detection of antibodies against BVDV. An overall seroprevalence of BVDV was estimated to be 48.6% (95% CI, 46.08%-50.98%). Considering the age group, a seroprevalence of 36.8% was detected in cattle <1 year, 41.0% in cattle between 1 and 2 years of age and 48.6% in cattle aged more than 2 years. Statistically significant differences ($p<0.001$) between age groups were reported. Seroprevalence of BVDV was significantly associated with geographical region ($P=0.033$). This is the first study on BVD in Libya and the results of this study would suggest that BVD is endemic in Libya.

Keywords: BVDV; risk factors; sero-prevalence; Libya

1. Introduction

Bovine viral diarrhea (BVD) is a viral disease that can be considered as a highly contagious disease reported in many domestic and wildlife animals. However, BVD as a name is affecting mainly cattle and can cause significant and huge losses for cattle owners [1]. BVD is caused by the BVD virus (BVDV) of the family Flaviviridae and belongs to the genus Pestivirus [2,3]. Two species of BVDV known as BVDV-1 and BVDV-2 as a result of their genetic and antigenic properties [4,5]. BVDV is back to 1949 when described for the first in New York City [6,7], however, another study is suggesting that BVDV has been circulating in cattle populations for long time [8]. The clinical manifestations of BVDV range from subclinical to severe disease with a high mortality rate. The clinical signs include gastrointestinal disorders, respiratory and reproductive symptoms. The clinical and characteristics of BVDV infection varies among animal populations, and accordingly, the type of infection depend on the multiple factors, infecting viral strain as well as age, reproductive status, and immunological status of the animal of infected animals [9]. Type of viral infection might be transient infection (TI) or persistent infection (PI).

 doi:10.20944/preprints202312.1947.v1

Libya is located in North Africa and bordered by the Mediterranean Sea to the North, Tunisia and Algeria to the west, Niger and Chad to the south, Sudan to the Southeast, and Egypt to the East. The livestock production systems in the region are characterized by extensive management, in some cases by nomadic or transhumant systems, which exacerbate the spread of diseases. Disease spread also stems from uncontrolled movements of ruminants due to trade, in particular imports from infected countries [10]. There are many TADs have been reported (stroked the country during the last couple of years, and meanwhile instability in this country has made the difficulties to implement surveillance and monitoring programs for emerging and re-emerging infectious diseases) in Libya with significant public health and socioeconomic impacts [10], consequently, there is a scarcity of epidemiological data regarding the BVDV, therefore, this study was undertaken to investigate the seroprevalence of BVDV in cattle in Libya, and to determine the risk factors associated with BVDV infection. There is a lack of comprehensive research on the prevalence and impact of BVDV in North Africa.

In 1972, the first BVDV sample was isolated from a calf suffering from severe enteritis. Most of the BVDV reports from Egypt are based mainly on the detection of the virus by isolation or detection of viral antibodies. Therefore, there are only a few reports that elaborate on the subtyping of circulating BVDV in animal populations.

A study by Ait-Oudhia et al. [11] conducted in Algeria found a high prevalence of BVDV antibodies in cattle, with 53.8% of animals testing positive. In another study, the prevalence was found to be about 59.9% [12]. In Tunisia, a study by Sassi et al. [13] found a lower prevalence of BVDV antibodies in cattle, with 11.8% of animals testing positive. The study noted that the prevalence of BVDV was higher in cattle raised for milk production than in those raised for meat production.

Similarly, a study by Fassi-Fihri et al. [14] conducted in Morocco found a moderate prevalence of BVDV antibodies in cattle, with 33.3% of animals testing positive.

While there is limited research on BVDV in North Africa, the available studies suggest that the disease is a significant concern for the cattle industry in the region. The high prevalence of BVDV antibodies in Algerian cattle [11], the higher prevalence in dairy cattle in Tunisia [13], and the risk factors identified in Moroccan cattle [14], all highlight the need for further research and effective management strategies to prevent and control BVDV in North Africa. Since there are no previous study on the BVDV in Libya, the objective of the study was conducted to investigate the level of sero-prevalence of BVDV in Libya and the associated risk factor.

2. Materials and Methods

2.1. Study area and study design

The study was conducted in seven Libya regions. Table 1 shows the seven regions with an estimated total number of cattle in each region.

Table 1. Distribution of cattle in seven Libyan regions.

No.	Region	Cattle population
1	Green mountain	88,000
2	Benghazi	13,000
3	Middle area	2,000
4	Zawia	17,000
5	Tripoli	29,000
6	West mountain	1,000
7	Sabha	4,000
Total		**154,000**

 doi:10.20944/preprints202312.1947.v1

2.2. Sampling collection and Questionnaire Survey

A total of 1599 serum samples were collected randomly from herds of cattle which belonging to seven Libyan regions. The structured well designated questionnaire was use collected all relevant data regarding the risk factors associated with infection (age group, sex, and Region).

2.3. Samples processing

Blood samples were collected from jugular vein. All samples are labeled for the identification purposes of each animal, and centrifugation of the serum stored until used. The samples were tested in Brescia, Italy, at IZSLER, an OIE/FAO reference laboratory by using screening ELISA assay for the detection of antibodies against BVDV.

After the wells were covered with antigen p80, the serum samples were incubated in them. Following the formation of the Ag- Ab complex, other steps including washing, adding conjugate, substrate, and stop solutions were carried out. After reading the optical density with an ELISA reader in a 450 nm wavelength for each sample, the S/N percentage (optical density /OD of the serum sample to OD of negative control) was calculated using the following formula:

$$S/N\% = ODSample \setminus ODNC \times 100$$

Samples were considered positive, doubtful and negative for (S/N%) ≤40, 40< to ≤ 50%, and > 50% respectively.

2.4. Statistical analysis

For each age group the prevalence and 95% confidence intervals (CI) were calculated using the Bayesian approach of Beta distribution. Chi-square in a univariable analysis was used for the association between the outcome variables including status of BVDV infection and risk factors. In addition, odds ratio was used to estimate the effect size as the association between the seroprevalence of BVDV and potential risk factors was analyzed using logistic regression. A p-value <0.05 was considered to be significant.

3. Results

In this study, the overall seroprevalence of BVDV was estimated to be 48.6% (95% Confidence interval (CL): 46.08%-50.98%). The results of the univariates analysis of independent variables were showed statistical differences, the result is significant at $p < .05$ as illustrated in Table 2.

Table 2. The Univariant analysis of BVDV seroprevalence and associated risk factors.

Risk factors	Animal tested	Animal affected (%)	DF	X2	P-value
Sex	1599	**48.5**	1	8.41	**0.004**
Male	198	38.9			
Female	1401	49.9			
Region			6	13.67	**0.033**
Green Mountain	478	49.1			
Benghazi	106	56.6			
Middle area	55	58.2			
Zawiyah	268	44.4			
Tripoli	519	49.1			
West Mountain	136	47.8			
Sabha	37	45.0			
Age group			2	12.94	**0.001**
<1 Year	185	36.76			

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 26 December 2023 doi:10.20944/preprints202312.1947.v1

1-2 Year	605	41
> 2 Year	809	48.6

Considering the age groups, high seroprevalence of BVDV were reported in age groups < 1 year 36.76% (95% CI, 29.81%-43.70%), 1-2 year 41% (95% CI, 37.07%-44.91%) and > 2 year 48.6% (95% CI, 45.13%-52.02%) (Figure 1), significantly (P=.001), the animal age factor was associated with BVDV infection. The results showed the seroprevalence of BVDV was significantly higher in adult animals than in young animals.

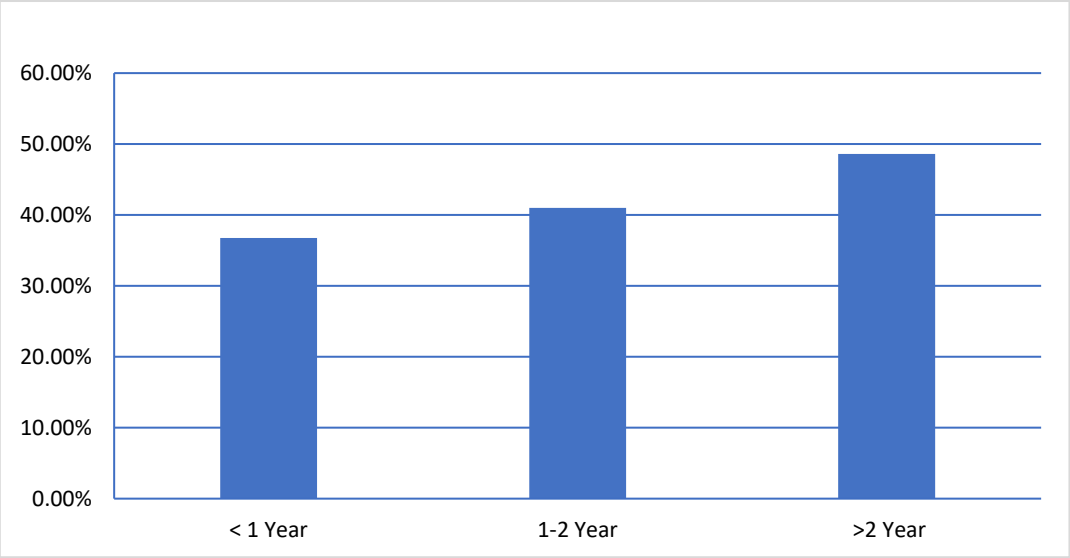

Figure 1. The seroprevalence of BVDV according to age groups.

The results showed a high BVDV seroprevalence of 38.9% (95% CI; 32.10%-45.68%) and 49.9% (95% CI; 47.27%-52.51%) were reported in males and females respectively (Figure 2). Significantly (P=.004), the gender factor was influenced by the seroprevalence of BVDV among the cattle.

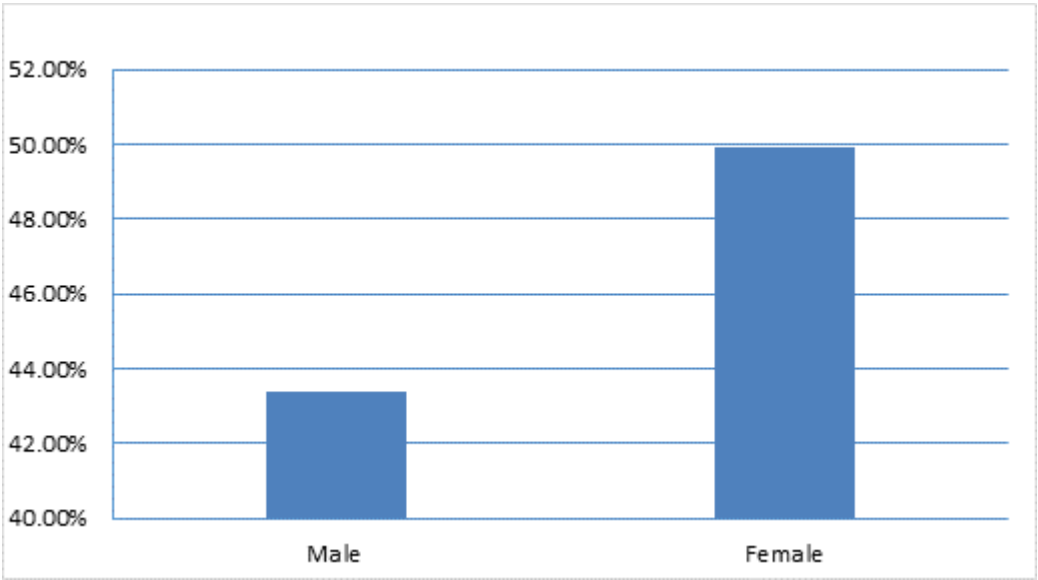

Figure 2. Seroprevalence of BVDV according to animal sex.

The results showed highest seroprevalence of BVDV in the middle area (58.2%; 95% CI, 45.15%-71.22%) and lowest seroprevalence in Sabha (Southern region) (27.03%; 95%, 12.72%-41.34%)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 26 December 2023 doi:10.20944/preprints202312.1947.v1

followed by the Zawiyh region (44.4%; 95% CI, 38.45%-50.35%) (Figure 3). Seroprevalence of BVDV was significantly associated with the geographical region (P= .033).

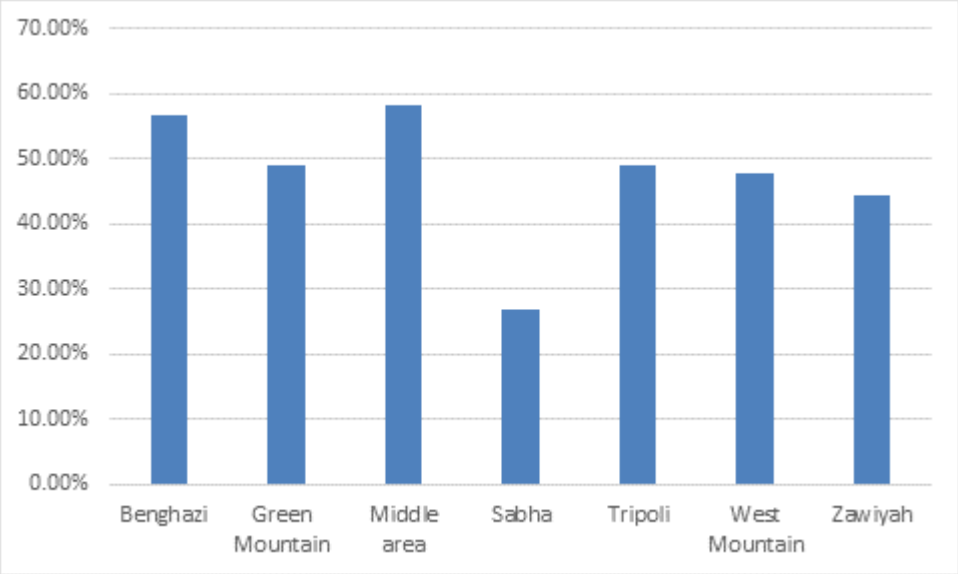

Figure 3. The seroprevalence of BVDV according to the Libyan geographical regions.

4. Discussion

The BVD is well known, described, and documented in the many kinds of literature conserving the almost North Africa and Mediterranean region. However, this is the first study to investigate BVDV among non-vaccinated cattle populations in Libya. Expectedly, this study reported a high seroprevalence of BVDV among dairy cattle in different parts of the country. In agreement to several studies reported high seroprevalence of BVDV in North Africa and Mediterranean regions [15]. In line with other similar studies indicating different seroprevalence between males and females with higher seroprevalence in females, the significant difference between sex (P=.004) found in this study could be explained by the fact that fewer number of males present in cattle herds. Herd's men sell the bulls after weaning resulting in higher numbers of older females than older males. The high seroprevalence of BVDV was estimated to be higher in adult animals than young. In agreement with several studies that reported frequently higher seroprevalence in adults [16,17]. Comparatively, the relatively high (36.76%) seroprevalence of BVDV in this study amongst young animals is considered another evidence of constant endemicity of BVD within the dairy cattle herds in the country. And in agreement with several studies from different parts of the world reported a significant association of BVDV infection with newborn animals are immunotolerant to and persistently infected (PI) with BVDV [18,19]. It's well-known that younger animal (calf) plays a crucial role in transmission and PI within bovine herds with BVDV. PI animals shed high titers of infectious BVDV from nasal and ocular secretions, urine, semen, colostrum/milk, and feces [20,21]. Unfortunately, our study was not designated to determine the PI in the pregnant dams and offspring calf among cattle populations. Consequently, the answer will remain unclear regarding the PI calf if could be playing a significant role in the epidemiology of BVDV within the cattle populations in Libya. Also, another question arises about impacts of BVDV on the reproductive performance among dairy cattle populations in Libya.

The present study reported the highest seroprevalence in the middle, Benghazi, and Green Mountain regions, followed by Western, Zawiyah, and Tripoli regions (Figure 3). Comparatively, the lowest seroprevalence of BVDV was reported in Sabha (Southern region). The difference in seroprevalence values that have been reported among regions might be attributable to (influenced by) animal dynamics, density, animal housing system, and distribution of cattle at the national regional level (herd size per farm). The high seroprevalence rate indicated that BVD is constantly

endemic in almost Libyan regions under the study. In spite, of the significant differences (P=0.033) in the BVDV seroprevalence values were demonstrated on the geographical level, conversely, seroprevalence was somewhat uniformly distributed in almost Libyan regions. And the highest seroprevalence values reported on the national regional level indicated a wide spatial distribution of BVDV infection among the most dairy cattle populations.

5. Conclusions

The present study revealed that BVDV infection is widespread among cattle populations in Libya. The results of this study would suggest that BVD is endemic in Libya, with a constant exposure to the infection of the animals during their life. More studies are still needed and one of the option s for the Libyan National Center for Animal Health to consider out of this study is to introduce vaccination against BVD as one of the control strategies to control this disease in Libya.

Author Contributions Conceptualization, H.E., I.B., I.E. and A.D.; methodology, H.E., I.B., E.B., S.G., A.M., I.E. and A.D.; validation, I.B., A.M., I.E. and A.D.; formal analysis, H.E., I.B., E.B., S.G., A.M. and A.D.; investigation, H.E., I.B., A.M., I.E. and A.D.; data curation, A.M., I.E. and A.D.; writing—original draft preparation, H.E., I.E. and A.D.; writing—review and editing, H.E., I.B., A.M., I.E. and A.D.; supervision, I.B. and A.D. All authors have read and agreed to the published version of the manuscript."

Funding: This research was funded by NCAH, Libya and Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER), Brescia, Italy.

Institutional Review Board Statement: Blood samples were collected from horses and dogs with the prior consent of their owners. This research was approved by the Department of Microbiology and Parasitology at the Faculty of Veterinary Medicine, University of Tripoli. Sample collection was carried out following approval from the Ethical Committee at the National Center for Animal Health in Libya (NCAH-15-2019).

Data Availability Statement: Data are available in the article. Any additional required data can be provided upon reasonable request from corresponding authors.

Acknowledgments: The authors would like to thank the personnel of the National Center for Animal Health who helped with sample collection.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Aguirre, I. M., Quezada, M. P., & Celedón, M. O. (2014). Antigenic variability in bovine viral diarrhea virus (BVDV) isolates from alpaca (Vicugna pacos), llama (Lama glama) and bovines in Chile. Veterinary microbiology, 168(2-4), 324–330.
2. Simmonds, P., Becher, P., Bukh, J., Gould, E. A., Meyers, G., Monath, T., Muerhoff, S., Pletnev, A., Rico-Hesse, R., Smith, D. B., Stapleton, J. T., & Ictv Report Consortium (2017). ICTV Virus Taxonomy Profile: Flaviviridae. The Journal of general virology, 98(1), 2–3.
3. Walz, Paul H., Manuel F. Chamorro, Shollie M. Falkenberg, Thomas Passler, Frank van der Meer, and Amelia R. Woolums.(2020). "Bovine Viral Diarrhea Virus: An Updated American College of Veterinary Internal Medicine Consensus Statement with Focus on Virus Biology, Hosts, Immunosuppression, and Vaccination." J Vet Intern Med 34 (5): 1690–1706.
4. Becher, P., Avalos Ramirez, R., Orlich, M., Cedillo Rosales, S., König, M., Schweizer, M., Stalder, H., Schirrmeier, H., & Thiel, H. J. (2003). Genetic and antigenic characterization of novel pestivirus genotypes: implications for classification. Virology, 311(1), 96–104.
5. King, A. M. Q., Lefkowitz, E. J., Mushegian, A. R., Adams, M. J., Dutilh, B. E., Gorbalenya, A. E., Harrach, B., Harrison, R. L., Junglen, S., Knowles, N. J., Kropinski, A. M., Krupovic, M., Kuhn, J. H., Nibert, M. L., Rubino, L., Sabanadzovic, S., Sanfaçon, H., Siddell, S. G., Simmonds, P., Varsani, A., … Davison, A. J. (2018). Changes to taxonomy and the International Code of Virus Classification and Nomenclature ratified by the International Committee on Taxonomy of Viruses (2018). Archives of virology, 163(9), 2601–2631.
6. OLAFSON, P., MacCALLUM, A. D., & FOX, F. H. (1946). An apparently new transmissible disease of cattle. The Cornell veterinarian, 36, 205–213.
7. Stalder, H., Bachofen, C., Schweizer, M., Zanoni, R., Sauerländer, D. and Peterhans, E., (2018). Traces of history conserved over 600 years in the geographic distribution of genetic variants of an RNA virus: Bovine viral diarrhea virus in Switzerland. PLoS One, 13(12), p.e0207604.

8. Chernick, A., & van der Meer, F. (2017). Evolution of Bovine viral diarrhea virus in Canada from 1997 to 2013. Virology, 509, 232–238.
9. OIE-World Organisation for Animal Health (2018): Bovine Viral Diarrhoea. Manual of Terrestrial Animals. P. 1075.
10. FAO. 2013. Transboundary animal diseases : diseases with a strong social and economic impact. Newsletter of the FAO sub regional office for North Africa, 3rd quarter 2013
11. Ait-Oudhia, K., Moulay, A., Yahiaoui, M., El Harrak, M., & Ait-Amrane, A. (2019). Prevalence and risk factors associated with bovine viral diarrhea virus (BVDV) in cattle in north-eastern Algeria. Tropical Animal Health and Production, 51(8), 2071-2076.
12. Guidoum KA, Benallou B, Pailler L, Espunyes J, Napp S, Cabezón O.(2020) Ruminant pestiviruses in North Africa. Prev Vet Med. 184:105-156.
13. Sassi, L., Ben Hassine, T., Jemli, M. H., Gharbi, M., & Gribâa-Dridi, L. (2016). Seroprevalence and risk factors associated with bovine viral diarrhea virus in dairy cattle in Tunisia. Tropical Animal Health and Production, 48(6), 1181-1186.
14. Fassi-Fihri, O., El Hicheri, K., Idrissi, A. H., & Loutfi, C. (2014). Séroprévalence de la diarrhée virale bovine au Maroc. Revue Scientifique et Technique de l'OIE, 33(3), 957-964.
15. Yılmaz, V. (2016). Prevalence of antibodies to bovine viral diarrhea virus (BVDV) in blood and milk serum in dairy cattle in Kars district of Turkey. Indian J. Anim. Res, 50(5),811-815.
16. Nigussie, Z., Mesfin, T., Sertse, T., Fulasa, T. and Regassa, F. (2010): Seroepidemiological Stud y of Bovine viral diarrhea (BVD) in three Agro-Ecological Zones in Ethiopia. Tropical Animal Health Production. 42: 319-321.
17. Handel, I.G., Willoughby, K., Land F., Koterwas, B., Morgan, K.L, Tanya, V.N. and Bronsvoort , B.M. (2011): Seroepidemiology of Bovine Viral Diarrhoea Virus (BVDV) in the Adamawa Region of Cameroon and Use of the SPOT Test to Identify Herds with PI Calves. Plos One. 6(7): 1-11.
18. Fulton, R.W., Hessman, B.E., Ridpath, J.F., Johnson, B.J., Burge, L.J., Kapil, S., Braziel, B., Kautz, K., and Reck, A. (2009): Multiple diagnostic tests to identify cattle with Bovine Viral Diarrhea Virus and duration of positive test results in persistently infected cattle. The Canadian Journal of Veterinary Research. 73: 117–124.
19. Akagami, M., Seki, S., Kashima, Y., Yamashita, K., Oya, S., Fujii, Y., Takayasu, M., Yaguchi, Y., Suzuki, A., Ono, Y., Ouchi, Y., & Hayama, Y. (2020). Risk factors associated with the within-farm transmission of bovine viral diarrhea virus and the incidence of persistently infected cattle on dairy farms from Ibaraki prefecture of Japan. Research in veterinary science, 129, 187–192.
20. Fulton, R. W., Ridpath, J. F., Ore, S., Confer, A. W., Saliki, J. T., Burge, L. J., & Payton, M. E. (2005). Bovine viral diarrhoea virus (BVDV) subgenotypes in diagnostic laboratory accessions: distribution of BVDV1a, 1b, and 2a subgenotypes. Veterinary microbiology, 111(1-2), 35–40
21. Kameyama, K., Konishi, M., Tsutsui, T. and Yamamoto, T. (2016) Survey for detecting persiste ntly infected cattle with Bovine Viral Diarrhea in Japan. The Journal of Veterinary Medical Science. 78(8): 1329–1331.